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Molecular Detection of *Vibrio* spp. in Fish and Shrimp from the Persian Gulf

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ABSTRACT: *Vibrio* species are the major seafood-borne bacteria that are frequently associated with the consumption of contaminated sea food. A total of 113 samples including 58 fish and 55 shrimps were studied for the possible contamination with *Vibrio* species. A biochemical protocol was applied for the identification of the *Vibrio* isolates and Polymerase Chain Reaction (PCR) was carried out to confirm the strains. The results indicated that 25 samples (22%) were contaminated with *Vibrio* species. Among *Vibrio* isolates, *Vibrio harveyi* was the species most frequently isolated (11.5%), followed by followed by *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus* and *V. mimicus*. *Vibrio cholerae* was not detected in the studied samples. The results of this study indicated that fish and shrimp from the Persian Gulf regularly contain pathogens that might affect the public health.

Keywords: *Fish, PCR, Persian Gulf, Shrimp, Vibrio spp.*

Introduction

Members of the genus *Vibrio* are motile, Gram-negative straight or curved rods (Roberts, 2001). They are facultative anaerobic chemo-organotrophs capable of both respiratory and fermentative metabolism (Roberts, 2001; Raissy *et al*., 2012). Some species, such as *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* are regularly linked to human food-borne infections caused by the consumption of raw, undercooked contaminated sea foods, but there are occasional reports of food-borne or waterborne infections caused by the environmental *Vibrio* or *Vibrio*-like species (Messelhausser *et al*., 2012). Other *Vibrio* species consist of *V. alginolyticus*, *V. harveyi*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, *V. mimicus* and *V. parahaemolyticus* that might cause disease in both aquatic animals and humans (Austin, 2012). Pathogenic *Vibrio* species such as *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* cause gastrointestinal illnesses or septicemia that might lead to fatal complications (Faruque and Nair, 2006; Janda *et al*., 1988). The mortality rate of *V. vulnificus* septicemia exceeds 50% and approaches 100% in cases of septic shock (Kumamoto & Vukich, 1998). Seafood products harvested from contaminated water or have been improperly preserved after harvesting are known to play an important role in the infections by bacteria (Mouzin *et al*., 1997). Occurrence of *Vibrio* in fish and shellfish has been reported in different countries including Iran (Janda *et al*., 1988; Jaksic *et al*., 2002; Hosseini *et al*., 2004; Rahimi *et al*., 2010) while the epidemiology of vibriosis, except *Vibrio cholera*, is essentially unknown in Iran. In this study the occurrence of *vibrio* spp. in fish and shrimp from the Persian Gulf and the possible harms for human is studied.

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Materials and Methods

- *Sample preparation*

A total number of 113 samples including 58 fish and 55 shrimps were collected from the local fish market during August 2010 to April 2011. The samples were transferred into cool boxes with an internal temperature of +2 to +4°C after collection and were processed within a short time after arrival.

- *Bacteriological Analysis*

The *Vibrio* spp. analysis took place according to the method described by Bockemuhl (1992). 25 g of the homogenized meat was added to 225 ml of alkaline peptone water (APW) and incubated at 37°C. The samples were subcultivated on Thiosulfate Citrate Bile Salts Sucrose agar (TCBS, BD Diagnostics, Heidelberg, Germany) and on modified Cellobiose Polymyxin-B Colistin agar (mCPC). After the incubation at 37℃ for 24h, the isolates were used for further screening tests including gram staining, oxidase and catalase tests, culture in SIM and TSI media and other biochemical tests as described by Hosseini *et al*. (2004).

- *DNA Extraction*

The genomic DNA was prepared using a standard DNA extraction method as described by Ausubel *et al*. (1987). The purity and quantity of genomic DNA in each sample was evaluated by measuring the optical densities at 260 and 280nm wavelength. The DNA concentration of each sample was adjusted to $50^{ng}/_{\mu l}$ for PCR.

- *PCR assay*

Two sets of oligonucleotide primers were used for specific identification of each *Vibrio* species. The primer sequences, targeting genes and amplicon sizes are listed in Table 1. The PCR reaction was performed in a 50 µl reaction system consisting of 2 µl of purified genomic DNA (50 ng/µl), 5 µl of 10×PCR buffer (100 mM Tris–HCl, pH 8.3, 500 mM KCl, 60 mM Mgcl2, 0.1% gelatin and 1% Triton X-100), 1 µl each of the primers (50 pmol/µl), 1 µl each of the 10 mM dNTPs, 0.2 µl units Taq DNA polymerase (5 units/µl) and 40 µl of sterile distilled water. The reactions were performed with a PTC-100 thermal cycler (Eppendorf, Harburg, Germany) with thermal cycling profile as is indicated in Table 1. Amplified products were separated by electrophoresis in ethidium bromide stained with 1.5% agarose gels at 90 V for 50 min. The gels were visualized and photographed with a UV transilluminator.

Results and Discussion

A total number of 113 samples consisting of 58 fish and 55 shrimps were analysed for *Vibrio* spp. using both biochemical tests and PCR. The results revealed that 25 samples (22.1%) including 18 fish and 7 shrimps contained *Vibrio* species. A total of 25 isolates were identified in this study where the most frequent species were *Vibrio harveyi* followed by *V. parahaemolyticus, V. vulnificus*, *V. alginolyticus* and *V. mimicus*. The number of samples contained *Vibrio* species are presented in Table 2.

Many studies show the presence of *Vibrio* species in aquatic animals such as fish (Messelhausser *et al*., 2012; Schmidt *et al*., 2000), shrimp (Raissy *et al*., 2012; Rahimi *et al*., 2012; Reboucas *et al*., 2011; Lhafi and Kuhne, 2009), lobster and crab (Raissy *et al*., 2012) and mussel (Lhafi and Kuhne, 2009).

In the present study, five *Vibrio* species including *V. vulnificus*, *V. parahaemolyticus, V. alginolyticus, V. harveyi* and *V. mimicus* were detected from the examined samples which is in agreement with the results of previous studies (Jaksic *et al*., 2002; Hosseini *et al*., 2004; Rahimi *et al*., 2010, Lhafi and Kuhne, 2009). According to the results, 29.3 % of the fish and 12.7 % of the shrimps samples examined revealed the presence of *Vibrio* species.

Among the *Vibrio* species detected in this

Table 1. Primer sequences, targeting genes and amplicon size of primers

Target species	Sequence(5'------ 3')	Amplicon Size (bp)	PCR program*	Targeting Gene	Reference
V. parahaemolyticus	GCAGCTGATCAAAACGTT GAGT ATTATCGATCGTGCCACTCAC	897 bp	a	*flaE*	(Tarr *et al*., 2007)
V. cholerae	AAGACCTCAACTGGCGGTA GAAGTGTTAGTGATCGCCAGAGT	248 bp	b	*sodB*	(Tarr *et al*., 2007)
V. vulnificus	GTCTTAAAGCGGTTGCTGC CGCTTCAAGTGCTGGTAGAAG	410 bp	c	*hsp*	(Tarr *et al*., 2007)
V. mimicus	CATTCGGTTCTTTCGCTGAT GAAGTGTTAGTG ATTGCTAGAGAT	121 bp	d	*sodB*	(Tarr *et al*., 2007)
V. alginolyticus	CGAGTACAGTCACTTGAAAGCC CACAACAGAACTCGCGTTACC	737 bp	e	*collagenase*	(Di Pinto *et al*., 2005)
V. harveyi	CTTCACGCTTGATGGCTACTG GTCACCCAATGCTACGACCT	235bp	f	*vhh*	(Maiti *et al*., 2009)

* PCR program: a, b, c, d: 35 times (92°C, 40 s; 57°C, 1 min; 72°C, 1.5 min); e: 35 times (94°C, 30 s; 57°C, 30 sec; 72°C, 1 min); f: 30 times (95°C, 1 min; 50°C, 1 min; 72°C, 1 min)

Table 2. The number of samples containing *vibrio* species

Sample	*V. vulnificus*	*V. parahaemolyticus*	*V. mimicus*	*V. alginolyticus*	*V. harveyi*
Scomberomorus commerson (24)	0	2	0	1	0
Otolithes ruber (15)	2	1	1	0	5
Scomberomorus guttatus (14)	1	0	1	0	3
Acanthopagrus latus (5)	0	0	0	1	0
penaeus semisulcatus (55)	0	1	0	1	5
Total (113)	3	4	2	3	13

study, *V. parahaemolyticus* has often been reported to cause gastro-intestinal problems following the consumption of contaminated seafood. In Japan, it has been reported to be responsible for one-fourth of all gastro-intestinal cases caused by food while in the USA, it has caused 14 outbreaks of food poisoning between 1971 and 1978 (Feldhusen, 2000). The occurrence of this species in Iran has been reported in shrimp, lobster and crab from the Persian Gulf (Raissy *et al*., 2012; Hosseini *et al*., 2004; Rahimi *et al*., 2010). The contamination of shrimp from the Persian Gulf with this species was reported in the order of 9.3% by Rahimi *et al*. (2010). Hosseini *et al*. (2004) reported four isolates of *V. parahaemolyticus* in 770 studied shrimp samples from the same place. In the present study, four samples (2.6%) were found to be contaminated with *V. parahaemolyticus*. Differences in contamination of seafood with *Vibrio* species might be related to the catching method, transportation condition, time of examination as well as seasonal and environmental conditions.

As an important human pathogenic bacterium, *V. vulnificus* has been associated with a small but increasing number of serious life-threatening conditions such as gastro-enteritis and wound infections which might become septicaemic (Mouzopoulos *et al*., 2008). The onset of symptoms is often abrupt, with a rapid progression to septic shock and thus death despite the intervention of antibiotics (Haq and Dayal, 2005). In the USA, *V. vulnificus* has been regarded as being responsible for most of the seafood related deaths since the first report in 1979 (Oliver, 2005). Indeed, a regular source of infection with this pathogen is the consumption of contaminated raw or under-cooked seafood (Drake et al., 2007). *V. harveyi* is proved to be a serious pathogen of shrimp and fish causing luminous bacterial disease in penaeid shrimp (Austin, 2010). In terms of human disease, *V. harveyi* has been recovered from wound infections, specifically from a leg wound resulting from a shark bite in USA (Pavia *et al*., 1989).

Conclusion

The results of this study indicated that vibrio species is a potential pathogen that

might be found in fish and shrimps caught in the Persian Gulf and might put human health at risk if consumed. The bacteria will be removed by using high cooking temperature, although the toxin might remain in the food stuff depending on the processing conditions.

References

Austin, B. (2010). Vibrios as causal agents of zoonoses. *Veterinary Microbiology*. 140, 310-317.

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Sideman, J., Smith, J. & Struhl, K. (1987). *Current Protocols in Molecular Biology*, Wiley, New York, pp. 35-40.

Bockemuhl, J. (1992). Vibrionaceae. In *Mikrobiologische Diagnostik*, Edited by Burkhardt, F., Georg Thieme Verlag, Stuttgart, pp. 102-108.

Drake, S. L., Depaola, A. & Jaykus, L. A. (2007). An overview of *Vibrio vulnificus* and *Vibrio parahaemolyticus*. *Comprehensive Reviews in Food Science and Food Safety*. 6, 120-144.

Faruque, S. M. & Nair, G. B. (2006). Epidemiology. In *The biology of vibrios,* Edited by Thompson, F.L. Austin B. & Swings, J., ASM Press, Washington D.C., pp. 385-398.

Feldhusen, F. (2000). The role of seafood in bacterial foodborne diseases. *Microbes and Infection*. 2, 1651-1660.

Haq, S. M. & Dayal, H. H. (2005). Chronic liver disease and consumption of raw oysters: a potentially lethal combination-a review of Vibrio vulnificus septicemia. *American Journal of Gastroentrology*. 100, 1195-1199.

Hosseini, H., Cheraghali, M., Yalfani, R. & Razavilar, V. (2004). Incidence of *Vibrio* spp. in shrimp caught off the south coast of Iran. *Food Control*. 15, 187-190.

Janda, J. M., Powers, C., Bryant, R. G. & Abbott, S. L. (1988). Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. Clinical Microbiology Reviews. 1, 245-267.

Jaksic, S., Uhitil, S., Petrak, T., Bazulic, D. & Gumhalter Karolyi, L. (2002). Occurrence of *Vibrio* spp. in sea fish, shrimps and bivalve molluscs harvested from Adriatic Sea. *Food Control*. 13, 491-493.

Kumamoto, K. S. & Vukich, D. J. (1998). Clinical infections of *Vibrio vulnificus*: a case report and review of the literature. *Journal of Emergency Medicine*. 16, 61-66.

Lhafi, S. K. & Kuhne, M**.** (2007). Occurrence of *Vibrio* spp. in blue mussels (*Mytilus edulis*) from the German Wadden Sea. *International Journal Food Microbiology*. 116, 297-300.

Messelhausser, U., Colditz, J., Tharigen, D., Kleih, W., Holler, C. & Busch, U. (2010). Detection and differentiation of *Vibrio* spp. in seafood and fish samples with cultural and molecular methods. *International Journal Food Microbiology*. 142, 360-364.

Mouzin, E., Mascola, L., Tormey, M. P. & Dassey, D. E. (1997). Prevention of *Vibrio vulnificus* Infections. Assessment of regulatory educational strategies. *Journal of American Medical Association*. 278, 576-578.

Mouzopoulos, G., Stamatakos, M., Tzurbakis, M., Batanis, G., Michou, E., Iannescu, R. & Safioleas, M. (2008). Lower extremity infections by *Vibrio vulnificus*. *Chirurgia*. 103, 201-203.

Oliver, J.D. (2005). Wound infections caused by *Vibrio vulnificus* and other marine bacteria. *Epidemiology and Infection*. 133, 383-391.

Pavia, A. T., Bryan, J. A., Maher, K. L., Hester, T. R. & Farmer, J. J. (1989). *Vibrio carchariae* infection after a shark bite. *Annals of Internal Medicine*. 111, 85-86.

Rahimi, E., Ameri, M., Doosti, A. & Gholampour, A. R. (2010). Occurrence of toxigenic *Vibrio parahaemolyticus* strains in shrimp in Iran. *Foodborne Pathogen and Diseases*. 7, 1107-1111.

Reboucas, R. H., De Sousa, O. V., Lima, A. S., Vasconcelos, F. R., De Carvalho, P. B. & Vieira, R. H. (2011). Antimicrobial resistance profile of *Vibrio* species isolated from marine shrimp farming environments (*Litopenaeus vannamei*) at Ceará, *Brazilil Environmental Resources*. 111, 21-24.

Roberts, R. J. (2001). *Fish pathology*. 3rd ed, W. B Sunders, London, pp. 311-315.

Schmidt, A. S., Bruun, M. S., Dalsgaard, I., Pederson, K. & Larsen, J. L. (2000). Occurrence of antimicrobial resistance in fish pathogen and environmental bacteria associated with four Danish rainbow trout farms. *Applied Environmental Microbiology*. 66, 4908-4915.